

REMARKS

In the Office Action dated September 11, 2007, Claims 38 and 42-47 are pending and under examination. Claims 38, 42-43 and 47 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Liu et al. (*J. Inorganic Biochemistry* 1998 Vol. 71, page 1-6) in view of Pisanti et al. (*Marine Pollution Bull* (1988) Vol. 19, page 328-333). Claims 44-46 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Liu et al. in view of Pisanti et al. and further in view of Gold et al. (U.S. Patent No. 6,242,246).

The undersigned attorney conducted a telephone interview with Examiner Cheu on November 16, 2007. Applicants, through the undersigned, wish to thank the Examiner for the courtesy and assistance extended to Applicants during the interview.

This Response addresses each of the Examiner's rejections. Applicants therefore respectfully submit that the present application is in condition for allowance. Favorable consideration of all pending claims is therefore respectfully requested.

Initially, Applicants respectfully submit that independent claims 38 and 47 have been amended to replace the term "metal atom" with "metal ion". Support for this recitation is found in dependent claim 39, and throughout the specification. No new matter is introduced.

Turning to the obviousness rejection based on Liu et al. in view of Pisanti et al., the Examiner maintains that Liu et al. teach a method of measuring fluorescence quenching of DNA bound fluorescence dye, e.g., increasing dissociation or inhibition of fluorescent dye bound to the DNA, by a sample containing a metal ion, i.e., copper (II) macrocyclic complexes. The Examiner states that Liu et al. teach that the presence of a copper (II) metal macrocyclic complexes would compete with binding the DNA molecule intercalated with the fluorescence ethidium dye. The Examiner contends that the binding constant of the copper is around 10^{-10}

(M^{-1}), which falls within the micromolar range as claimed. The Examiner acknowledges that Liu et al. do not explicitly teach detecting the inhibition or dissociation of the dye on the DNA as an indication of the presence of a metal in an environmental sample. However, the Examiner alleges that Pisanti et al. teach that the presence of metals in the ecosystem, e.g., ocean or rivers, is of great concern. Therefore, the Examiner alleges that it would have been obvious to one skilled in the art, at the time the present invention was made, to combine the teachings of Liu et al. and Pisanti et al. in order to measure the presence of copper in an aquatic sample.

Applicants previously submitted that in contrast to the present invention, Liu et al. do not disclose or suggest how to directly detect a metal atom that exists in an environmental sample in the absence of any macrocyclic compound. In fact, Liu et al.'s method requires an initial step of preparing and purifying copper (II) macrocyclic complexes (CuL) (see Fig. 1 and page 2, column 2, lines 2-5); and such complexes are then used in fluorescence quenching of DNA bound to ethidium bromide.

However, the Examiner has responded to Applicants' argument by stating that the term "comprising" in claim 38 does not exclude other materials such as the copper (II) macrocyclic complex taught by Liu et al. The Examiner state that "conducting the assay taught by Liu et al., as long as quenching is detected, this observation would reflect the amount of metal copper (II) in the environment" (Page 5, middle paragraph of the Action), apparently suggesting that copper ion in an environmental sample could be complexed with a macrocyclic compound.

Applicants respectfully reassert that the presently claimed methods are not obvious in view of Liu et al. and Pisanti et al. In particular, there would have been no motivation, or even any reason, for those skilled in the art to combine the teachings of the two references. In support

of this position, Applicants provide herewith a Declaration of Professor Cris dos Remedios (Exhibit A).

As discussed in the Declaration, the primary reference to Liu et al. describes fluorescence quenching of DNA bound to ethidium bromide by two specific Cu (II) macrocyclic complexes in order to estimate binding constants between the complexes and the DNA. As indicated by Liu et al., the Cu (II) macrocyclic complexes were prepared and purified by the literature methods described by Truer and Holm (1972) (attached to the Declaration as Exhibit II) and by Liu et al. (1993). Truer and Holm (1972) describe that (see for example, page 4530, first column, lines 4-8) naturally occurring macrocycles are usually of the 6-6-6-6 or 6-6-6-5 type. In contrast, as stated in the Declaration, paragraph 4, the Cu (II) complexes studied by Liu et al. contain alternating 6- and 5-membered rings, *i.e.* are of the 6-5-6-5 type. Therefore, Liu et al. disclose fluorescence quenching of DNA bound ethidium bromide by two specific non-natural Cu (II) complexes, which were prepared for the specific purpose of estimating binding constants. Importantly, as stated in the Declaration, paragraph 5, the non-natural Cu (II) complexes disclosed by Liu *et al.* normally would not be expected to exist in an environmental sample.

Therefore, Applicants respectfully submit that those skilled in the art would not have had any motivation to conduct an assay by applying a nucleic acid molecule intercalated with a fluorescent dye, as allegedly taught by Liu et al., to an environmental sample, because the non-natural Cu (II) complexes disclosed by Liu et al. would not exist in an environmental sample, and the assay disclosed by Liu et al. would simply not be operative for detecting metal ions in the sample absent a macrocyclic complex.

Applicants further observe that Liu et al. disclose the displacement of ethidium bromide from DNA by a copper-polypyridyl complex, leading to fluorescence quenching. As a result of the high affinity of the macrocyclic compound for metals and the high affinity of the macrocyclic compound-metal complex for DNA, the method of Liu et al. is very sensitive and will detect very low, nontoxic levels of metal ions. As submitted in the previous Response, the high stability of the complex between metal and macrocyclic polypyridyl is described by Bazzicalupi et al., *Dalton Trans.* 591-597 (2004) (copy attached hereto again as **Exhibit B**), in which the stability constant K_a of complexes between Hg, Cd and Zn and similar microcyclic compounds was calculated to be in the order of 10^{16} M. Therefore, Applicants submit that the teachings of Liu et al. would not have provided those skilled in the art any motivation to apply the method disclosed therein in order to detect toxic levels of metal ions in environmental samples. Rather, those skilled in the art would have been discouraged to apply Liu's method because of its extremely high sensitivity and ability to detect nontoxic levels of metal ions, leading to false positive results.

In contrast, the present invention is directed to detecting micromolar amounts of a toxicant that is a metal ion in an environmental sample, i.e., metals that exist in an environmental sample at toxic levels. Applicants respectfully submit that contrasting to the method of Liu et al., the instantly claimed method, which does not employ any macrocyclic compound, is more discriminating so that low or non-toxic levels of metal ions are not detected.

In view of the foregoing, Applicants respectfully submit that the Examiner has failed to establish a *prima facie* case of obviousness based on Liu et al. in combination with Pisanti et al. Therefore, the rejection under 35 U.S.C. §103(a) as allegedly unpatentable over Liu et al. in view of Pisanti et al. is overcome and withdrawal thereof is respectfully requested.

Claims 44-46 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Liu et al. in view of Pisanti et al. and further in view of Gold et al. (U.S. Patent No. 6,242,246).

As discussed above, Applicants respectfully submit that the combination of the teachings of Liu et al. and Pisanti et al. does not render the present invention obvious. Furthermore, Applicants submit that the reference to Gold et al. does not obviate the deficiencies of the references to Liu et al. and Pisanti et al. Thus, the Examiner has failed to establish a *prima facie* case of obviousness based on these references.

Therefore, the rejection of Claim 44-46 under 35 U.S.C. §103(a) as allegedly unpatentable over Liu et al. in view of Pisanti et al., and further in view of Gold et al., is overcome, and withdrawal thereof is respectfully requested.

In view of the foregoing amendments and remarks, it is firmly believed that the present application is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,



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Encls.: Exhibit A (Declaration and Exhibits I-II attached thereto); and Exhibit B.